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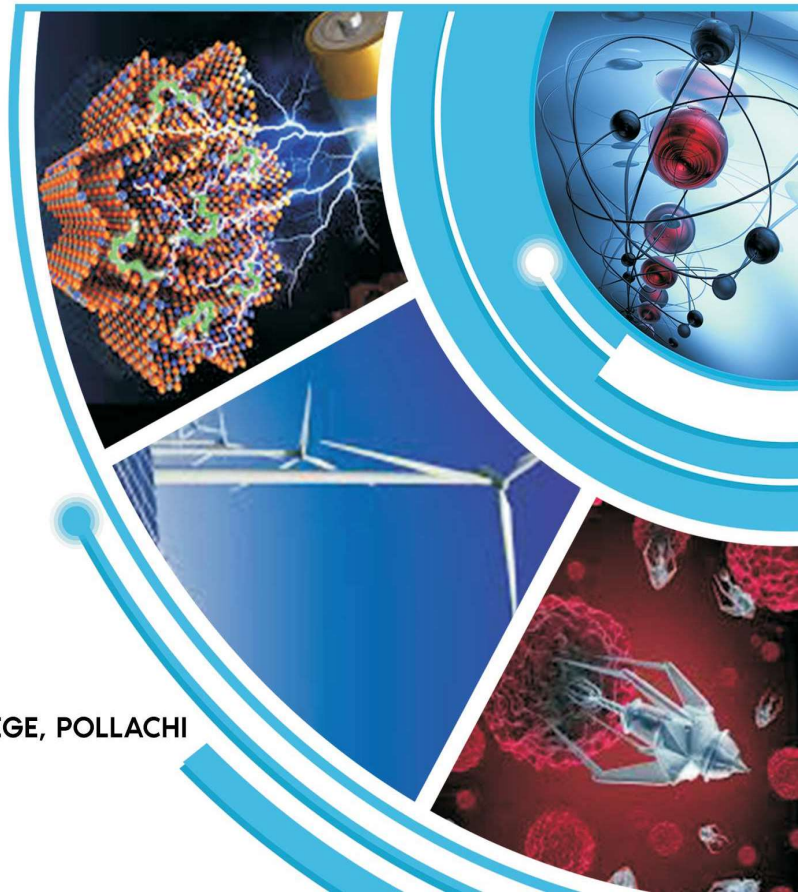

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CHEMICAL COMPOSITION AND *INVITRO* ANTICANCER ACTIVITY OF *OCIMUM SANCTUM* AGAINST MOLT – 3 CANCER CELL LINE

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ABSTRACT

The present study examines the nature of phytoconstituents and *in vitro* anticancer activity of essential oil of *Ocimum Sanctum* leaves. GC-MS analysis of *Ocimum Sanctum* essential oil revealed the presence of twelve compounds. In the present study, the major chemical constituents are cis-6-(1,1-dimethylethyl)-2,3,4,4a,5,6,7,8-octahydro-1-naphthalenol-4-nitrobenzoate (42.29%), 1,4,8-CYCLOUNDECATRIENE, 2,6,6,9-TETRAMETHYL-, (E,E,E) (1.85%), 3-Hydroxy-5-isopropyl-3,8-dimethyl-1,2,3,4,7,8,9,10-o Ctahydronaphthalene (1.46%). The *in vitro* anticancer activity was tested against MOLT - 3 blood cancer cell line using MTT assay. The essential oil of *Ocimum Sanctum* showed dose dependent anticancer activity. The IC_{50} value was 13µg/ml. The result revealed that *Ocimum Sanctum* plant has most potent anticancer activity.

Keywords: *Ocimum Santum*, GC-MS, *in vitro* anticancer activity, MOLT - 3 cell line, MTT assay

INTRODUCTION

Ocimum sanctum (tulsi) belongs to the family *Lamiaceae*, consists of about 160 species. It is well discussed in Ayurveda as healing system.[1] *Ocimum sanctum* has specific aromatic odour because of the presence of essential or volatile oil, mainly concentrated in the leaf. This aromatic volatile oil mainly contains phenols, terpenes and aldehydes. The oil extracted from seeds is called fixed oil and mainly composed of fatty acids. Besides oil, the plant also contains alkaloids, glycosides, saponins and tannins. The leaves contain ascorbic acid and carotene as well. The present day information about the chemical properties is based on the various studies that have been done in different parts of the world[2] and it is likely that chemical constituents may be varying due to edaphic and geographic factors.[3] Many authors reported pharmacological activity of *Ocimum Sanctum* plant like analgesic activity, Anticataract activity, anti-arthritic activity, Anti-diabetic activity and Anticoagulant activity.[4-8]

There is limited work on anticancer activity. So our main objective of the present work is to evaluate the phytoconstitutions and the anticancer activity against MOLT -3 blood cancer cell line using MTT Assay.

MATERIALS AND METHODS

Plant Material

Fresh leaves of *Ocimum Sanctum* were collected from area near Pollachi,Tamilnadu, South India between the periods of august to September. The plant sample was authenticated by Department of Botany, NGM College, Pollachi, Coimbatore.

Isolation of Essential Oil

The fresh leaves of *Ocimum Sanctum* (200g) were collected and washed with distilled water. The essential oil was isolated by using Clevenger's apparatus on 4hrs. The isolated fraction showed two distinct layers-an upper oily layer and a lower aqueous layer. Both the layers were separated and the moisture from the oily layer was removed by adding anhydrous sodium sulphate. The collected essential oil was transferred into a dark glass bottle and kept at a temperature of 4 °C prior to GC-MS analysis.

GC-MS Analysis

GC-MS analysis of the phytoconstituents of *Ocimum sanctum* was carried out using thermo GC –trace ultra-version: 5.0 coupled with thermo MS DSQ II instrument. Compounds were separated on DB-35, MS capillary standard non – polar column (30m × 0.25 mm), film thickness 0.25µm. Helium was used as the carrier gas and the temperature programming was set with initial oven temperature at 700C and held for 2 minutes and the temperature of the oven was raised to 2600C for 10min and raised 60C/MIN and final temperature was 3500C for 10 min. The sample of 100mL was dissolved in 1mL of acetone and injected with split less mode. Mass spectra were recorded over 50-500 amu range with electron impact ionization energy 70 eV, while injector and MS transfer line temperature were set at 2800C respectively.

Identification of phytoconstituents

The components were identified by comparison of their mass spectra with those of National Institute of Science and technology (NIST) mass spectral library version 2.0d, as well as on their comparison of their retention time either with those of authentic compounds or with their literature values.

***In vitro* anticancer activity by MTT assay**

The *in vitro* anticancer activity of essential oil of *Ocimum Sanctum* leaves were tested against MOLT – 3 blood cancer cell line using MTT assay. The essential oil was diluted to different concentrations via 5µl, 25µl, 50µl, 75µl, 100µl. The cell viability of cancer cells after inhibition was noted.

MTT assay

3-[4,5-dimethylthiazol-2-yl]2,5-diphenyltetrazolium bromide (MTT) is a yellow water soluble tetrazolium salt. A mitochondrial enzyme in living cells, succinate-dehydrogenase, cleaves the tetrazolium ring, converting the MTT to an insoluble purple formazan. Therefore, the amount of formazan produced is directly proportional to the number of viable cells. After 48 hours of incubation, 15µl of MTT (5mg/ml) in phosphate buffered saline (PBS) was added to each well and incubated at 37ºC for 4 hours. The medium with MTT was then flicked off and the formed formazan crystals were solubilized in 100µl of DMSO and then measured the absorbance at 570nm using micro plate reader. The % cell inhibition was determined using the formula

$$\% \text{ cell inhibition} = 100 - [\text{Abs (sample)}/\text{Abs (control)}] * 100$$

IC_{50}value was determined by Nonlinear regression graph was plotted between % cell inhibition and $\log_{10}$ concentration.

RESULTS AND DISCUSSION

GC-MS Analysis

The GC – MS is used to identify the compounds like Terpenes, hydrocarbons, fatty acids and branched chain hydrocarbons. The GC-MS analysis of the essential oil of *Ocimum Sanctum* leaves showed the presence of 12 chemical constituents. The compounds were characterized and identified by comparing the GC-MS spectra of the constituents with the NIST library along with the parameters which includes the retention time, peak area, molecular weight. Among the 12 compounds identified the most popular compound is cis-6-(1,1-dimethylethyl)-2,3,4,4a,5,6,7,8-octahydro-1-naphthalenol-4-nitrobenzoate (42.29%), 1,4,8-CYCLOUNDECATRIENE, 2,6,6,9-TETRAMETHYL-, (E,E,E) (1.85%), 3-Hydroxy-5-isopropyl-3, 8-dimethyl-1,2,3,4,7,8,9,10-o Ctahydronaphthalene (1.46%). The GC-MS chromatogram is shown in the figure 1 and the characterized compounds were shown in table 1.

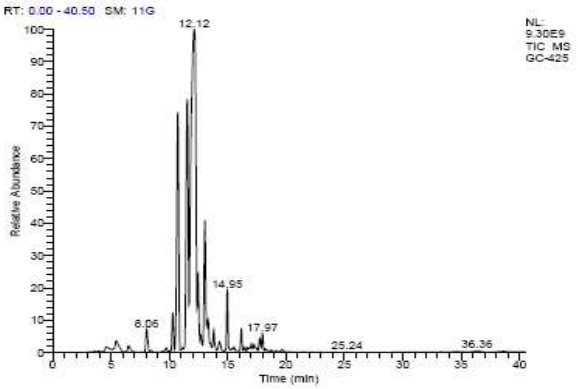

Figure 1: GC-MS chromatogram of essential oil of *Ocimum Sanctum* leaves

Table 1: Chemical composition of essential oil of *Ocimum Sanctum* leaves

S.NO	COMPOUND NAME	RETENTION TIME	AREA
1.	Bicyclo[2.2.1]heptan-2-one,1,7,7-trimethyl-, (1S)-	8.06	1.68
2.	Isocaryophillene	11.14	0.08
3.	Aromadendrene	11.14	0.08
4.	cis-6-(1,1-dimethylethyl)-2,3,4,4a,5,6,7,8-octahydro-1-naphthalenol-4-nitrobenzoate	12.12	42.29
5.	1,4,8-CYCLOUNDECATRIENE,2,6,6,9-TETRAMETHYL-, (E,E,E)	12.47	1.85
6.	2-Isopropenyl-4a,8-dimethyl-1,2,3,4,4a,5,6,7-octahydro naphthalene	12.71	0.23
7.	4à-Hydroxymorph-5-ene [(1R,4R,7R,10S)-4-hydroxy-7-(1-methylethyl)-4,10-dimethylbicyclo[4.4.0]dec-5-ene]	14.29	0.71
8.	1-vinyl-2-(1'-ethynyl-1'-hydroxyethyl)cyclohexane	16.75	0.27
9.	10-epi-ç-eudesmol	17.04	0.40
10.	3-Hydroxy-5-isopropyl-3,8-dimethyl-1,2,3,4,7,8,9,10-o Ctahydronaphthalene	17.97	1.46
11.	6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydro-naphthalen-2-ol	19.17	0.09
12.	5,8-Dihydro-3,4-diphenyl-5,8-methanoquinoxaline	36.38	0.12

***In vitro* anticancer activity by MTT assay**

MTT assay is a reliable method for screening the anticancer activity by measuring the cell viability. The present study examined the *in vitro* anticancer activity of essential oil of *Ocimum Sanctum* leaves against MOLT – 3 blood cell line using MTT assay. The incubation with different concentration of the essential oil affected the viability of MOLT – 3 cell line in dose dependent pattern. The extract was diluted to 5µl, 25µl, 50µl, 75µl, 100µl. The IC_{50} value was 13µg/ml and results showed that the posses most potent anticancer activity.

Table 2: % Cell inhibition in different concentration of essential oil of *Ocimum Sanctum* leaves.

S.No	Concentration (µg/ml)	% of cell inhibition
1	5	39.2
2	25	60.0
3	50	66.1
4	75	69.2
5	100	78.3

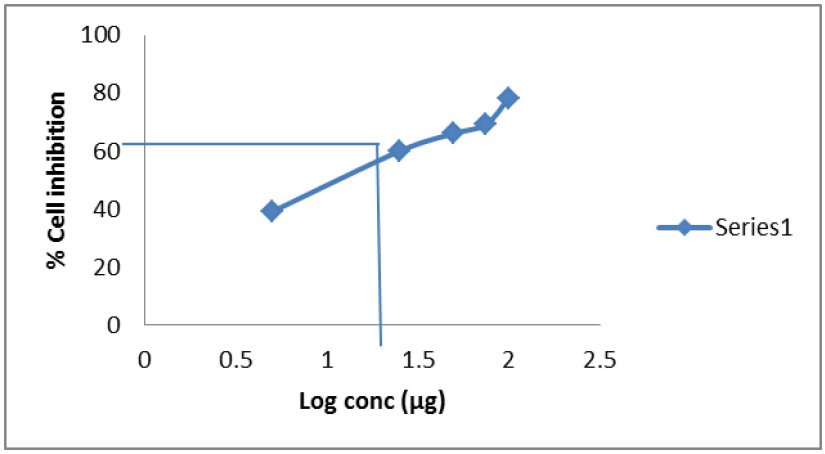

Figure 2: The IC_{50} value is determined by the plot of concentration Vs % incubation

Comparison between the anticancer activities of *Ocimum Sanctum* essential oil on MOLT – 3 cell line with KB Oral cancer cell line

When the KB Oral cancer cell line was used, absorbance values showed lower than the control cells indicate a reduction in the rate of cell proliferation. Conversely, a higher absorbance rate indicates an increase in cell proliferation. Evidence of cell death may be inferred from morphological changes. The *Invitro* anticancer activity of *Ocimum Sanctum* essential oil on KB Oral cancer cell line was showed that Severe cytotoxicity with 10.0µg/ml concentration in MTT assay. The anticancer activity of *Ocimum Sanctum* essential oil on MOLT – 3 cell line shows that Mild to Severe cytotoxicity with concentration 20µg/ml in MTT assay and the IC_{50} value is 13.89µg/ml.

So the resulting of *Invitro* anticancer activity of *Ocimum Sanctum* essential oil on MOLT – 3 cell line shows that some less cytotoxicity with compared to KB Oral cancer cell line.

CONCLUSION

The present study reveals that the essential of *Ocimum Sanctum* leaves has shows that most potent anticancer activity against MOLT – 3 blood cancer Cell line. The isolation of active phytoconstituents is under process.

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